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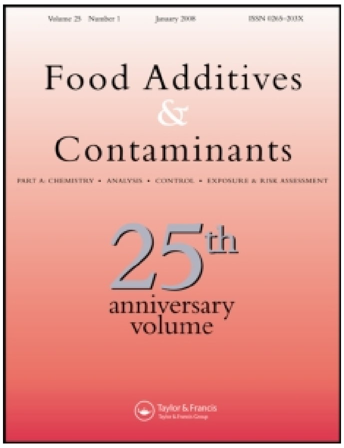

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Delivery systems for biological control agents to manage aflatoxin contamination of pre-harvest maize

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Delivery systems for biological control agents to manage aflatoxin contamination of pre-harvest maize

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While soil application of a competitive non-toxigenic *Aspergillus flavus* strains is successful in reducing aflatoxin contamination in certain crops, direct application to aerial reproductive structures could be more effective for maize. A sprayable, clay-based water-dispersible granule formulation was developed to deliver non-toxigenic *A. flavus* strain K49 directly to maize ears. The efficacy of the K49 water-dispersible granule in mitigating aflatoxin in maize (*Zea mays* L.) was evaluated. Field studies were conducted to compare K49 colonization and effectiveness in reducing aflatoxin contamination when applied either as a soil inoculant or as a directed spray in plots infested with toxigenic strain F3W4. Fifty percent of non-toxigenic *A. flavus* was recovered from non-treated controls and from plots soil inoculated with K49 on wheat. In spray treatments with formulated or unformulated K49 conidia, over 90% of *A. flavus* recovered was non-toxigenic. Soil-applied K49 reduced aflatoxin contamination by 65% and spray applications reduced contamination by 97%. These findings suggest direct spray application of non-toxigenic *A. flavus* strains may be better than soil inoculation at controlling maize aflatoxin contamination and that a water-dispersible granule is a viable delivery system for maintaining viability and efficacy of the biological control agent, K49.

Keywords: aflatoxin; *Aspergillus flavus*; biological control; formulation; spray application; maize (*Zea mays* L.)

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Introduction

Many fungi produce secondary metabolites that are not necessary for their growth or for reproduction. When toxic to humans or livestock, these metabolites are classified as mycotoxins. Four of the most important mycotoxin-producing fungal genera are *Aspergillus*, *Fusarium*, *Penicillium*, and *Alternaria* (Council for Agriculture Science and Technology (CAST) 2003). These fungi produce mycotoxins that could adversely affect the quality and supply of various food and feed commodities including maize, cottonseed, cereal grains, peanuts, and tree nuts.

Mycotoxins are estimated to cause an overall loss of US $5 billion annually for US and Canadian feed and livestock industries and aflatoxin produced by *Aspergillus* spp. is of greatest concern (Robens and Cardwell 2005). Aflatoxins produced by both *A. flavus* and *A. parasiticus* are prevalent in food and feed as contaminants (Payne 1992). Of the four aflatoxins (B_1, B_2, G_1, and G_2), aflatoxin B_1 (AFB_1) is regarded as the most potent and prevalent (International Agency for Research on Cancer–World Health Organization (IARC-WHO) 1993). Commodity contamination incidents are most frequently linked to *Aspergillus flavus* (Diener et al. 1987; Payne 1992; Horn 2003). The fungus is capable of growing over a wide temperature and water activity range of 10–43°C and 0.820–0.998, respectively (Food and Agriculture Organization of the United Nations/International Atomic Energy Agency (FAO/IAEA) 2001). Pre-harvest aflatoxin contamination can be exacerbated by drought conditions, mechanical injury, and pest damage (Payne 1992; Dowd 2003; Bruns and Abbas 2006; Abbas et al. 2007).

Current maximum aflatoxin level permissible in human food and animal feed is $20\,\mu g\,kg^{-1}$ in the USA (CAST 2003; Van Egmond et al. 2007). Although the presence of mycotoxins on agricultural commodities is unavoidable, the level of these contaminants can be controlled with good agronomic practices (Bruns and Abbas 2006). Several pre-harvest aflatoxin-management strategies have been proposed (Betrán and Isakeit 2004) with varying degrees of success. One promising control strategy is biological control using competitive, non-toxigenic *A. flavus* (Dorner 2004). Brown et al. (1991) demonstrated that aflatoxin levels could be suppressed by direct inoculation via mechanical wounds to maize ears. In contrast to the direct delivery strategy of Brown et al. (1991), the more common approach is indirect and involves soil inoculation with

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non-toxigenic strains of *A. flavus* initially cultured on cereal grains. While on the grains, the non-toxigenic strain may sporulate profusely, get dispersed by wind or water and compete with endemic toxigenic strains for resources, which would lead to a reduction of aflatoxin levels. This strategy has been successful in peanuts (Dorner et al. 1992), cotton (Cotty 1994) and even maize (Dorner et al. 1999). A similar soil applied inoculation strategy was implemented for Mississippi Delta maize production (Abbas et al. 2006; Abbas and Zablotowicz 2008) in which a non-toxigenic strain of *A. flavus*, K49 resulted in significant reduction of aflatoxin contamination in four years of field trials and exhibited good colonization potential. While soil inoculation is currently the main aflatoxin control strategy, several approaches to enhance performance of crop-enhancing fungi by novel formulations have been developed for delivering bioherbicides (Greaves et al. 1998), soil-applied beneficial fungi (Plenchette and Strullu 2003), and foliar application of entomopathogenic fungi (Lee et al. 2006).

The current field study was conducted to investigate the potential of a newly developed water-dispersible granule formulation that directly delivers K49 to maize ears at reducing aflatoxin contamination in pre-harvest maize. Treatments with formulated and unformulated conidia of K49 applied as a spray were compared with soil inoculation treatments and the effects on colonization potential and aflatoxin levels in field maize were determined.

Materials and methods

Aspergillus flavus *strains*

Non-toxigenic *A. flavus* strain K49 (NRRL 30797) and toxigenic strain F3W4 (NRRL 30796) were maintained on silica gel at 4°C and verified for appropriate phenotypic characteristics, aflatoxin profile, sclerotia formation, and colony morphology and conidia formation before initiation of studies (Abbas et al. 2006).

Water-dispersible granule materials and preparation

Satintone 5HB, a calcined kaolin clay, was provided by Englehard Corporation (Iselin, NJ, USA) for use as a carrier in the water-dispersible granule formulation. Sodium carboxymethylcellulose (Nilyn XL 90), provided by FMC Corporation (Philadelphia, PA, USA), was used as a binder in addition to trehalose (Cargill, Inc., Minneapolis, MN, USA). Trehalose, used as a multifunctional formulant, was included in the formulation to serve as an osmoprotectant, post-application sticker and potential nutrient source for K49.

The composition of the dry ingredients in the formulation was 76% Satintone 5HB, 4% Nilyn XL 90, and 20% trehalose. Dry ingredients were mixed until visually homogeneous in a food processor before mixing in 510 ml 0.1% (w/v) peptone solution containing 5% of the total dry amount of trehalose and conidia of K49. Conidia were harvested from 7-day-old malt extract agar plates with small aliquots of a 0.1% peptone solution. Control granules without the technical material were prepared and processed as described above.

In separate batches the above mixtures were extruded in a pan granulator (LCI Corporation, Charlotte, NC, USA) equipped with either a 1.2 or 2.0 mm die and dried under vacuum to a water activity of approximately 0.30. The 2.0 and 1.2 mm granules will be referred to as product 1 and product 2, respectively. The granules were stored at 4°C for approximately 330 days and the survival of the *A. flavus* propagules were determined occasionally by plating on semi-selective media. Triplicate samples were homogenized in water agar (0.2% w/v) using reciprocal shaking (30 min, 100 strokes per min), serially diluted and plated on modified dichloronitroaniline rose bengal media (MDRB) (Horn and Dorner 1998).

Conidia and solid inoculum preparation

For unformulated conidia inoculum production, stock cultures were transferred to 40 potato dextrose agar (PDA) plates and incubated for 7 days at 28°C in continuous darkness. Conidia and mycelium were scraped off the plate with aqueous Tween 20 (0.2% w/v). Vegetative fungal structures were removed from the conidia suspension by filtering through two layers of cheesecloth. The density of conidia was determined using a haemocytometer and adjusted to a final concentration of 4.1×10^6 conidia ml^{-1}.

Wheat was used as the inoculant carrier for soil inoculation as described by Abbas et al. (2006). Wheat seed was hydrated in water overnight, drained, and autoclaved in polypropylene bags (1 kg/bag with 200 ml water) for 1 h at 121°C. Inocula of *A. flavus* were 3 cm^2 sections of fresh 5-day-old PDA cultures per bag. After 48 h of incubation at 35°C, the wheat was fully colonized; and this product was homogenized by manual shaking and stored at 4°C until used for field trials.

Field colonization pin-bar assay

A pin-bar inoculation technique (Windham et al. 2003; Abbas et al. 2008) was used to determine the relative colonization ability of an unformulated conidial suspension of K49 compared with the water-dispersible granule formulation of K49 in 2005 field trials conducted at Stoneville and Elizabeth, Mississippi. The glyphosate-resistant hybrid (Garst 8270RR) was

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used in Stoneville, and a hybrid expressing the *Bacillus thuringiensis* endotoxin gene *Cry 1Ab* (Agrigold A6333Bt) was used in Elizabeth trials. Single rows of maize ears were inoculated at 25 days after mid-silking (dent kernels development), with double rows of non-inoculated maize as the buffer between treated rows. Maize ears (100 per treatment) were inoculated separately with either formulated ($15\,g\,l^{-1}$) or unformulated conidia of K49 (5×10^6 conidia ml^{-1}) at mid-silking stage using a pin-bar (three 100 mm-long rows of twelve sewing needles mounted on a wood block with 6 mm of the points exposed). Pin-bars were dipped in conidial suspensions, and the bars were pressed into the ear. At 2, 5, 7, 9, 12, and 14 days after treatment, ten inoculated ears were randomly harvested per treatment and the total number of kernels in the inoculated zone and the number of colonized kernels were determined based on counting and visual assessment of fungal growth on individual kernels (Abbas et al. 2006, 2008).

Field experiment aflatoxin control and strain establishment

A field study was conducted at Stoneville in 2006 to evaluate the efficacy of foliar applications of the biological control non-toxigenic strain K49 to reduce colonization by toxigenic isolates of *Aspergillus flavus* and reduce aflatoxin contamination. Maize (DK C69-70RR) was planted on 14 April 2006 on a Dundee silt loam soil in Stoneville. An experimental design of a randomized complete block design with six treatments replicated in three blocks was used. Each experimental unit consisted of three 9.36 m rows (1.06 m wide). Treated rows (centre 25–30 plants) were separated by two non-treated buffer rows to minimize cross-contamination. The six treatments consisted of (1) non-treated control; (2) soil inoculated with a wheat inoculant of the toxigenic *A. flavus* strain F3W4 ($20\,kg\,ha^{-1}$); (3) soil inoculated with a wheat formulation of K49; (4) soil inoculated with both F3W4 and K49 wheat formulations; (5) suspension of K49 extruded granules; and (6) suspension of spray suspension of freshly harvested K49 conidia.

On 26 June (at early silking stage), a wheat inoculant of the toxigenic strain F3W4 and the non-toxigenic strain K49 was applied to appropriate plots at a rate of $20\,kg\,ha^{-1}$. Four days later, the spray inoculants (treatments 5 and 6) were applied using a hand-held sprayer. The spray consisted of 56 g of formulated product suspended in 4 litres of 0.2% w/v Tween 20, applied at a rate of approximately 600 ml per plot. The spray was directed to the upper one-third of plants, targeting the primary ears. At physiological maturity (17 August), all ears in a 6 m length from the centre of the treated row were hand-harvested, dried, shelled and ground as described by Bruns and Abbas (2006).

Aflatoxin determination and Aspergillus *recovery*

Aflatoxin concentration was quantitatively determined using commercial enzyme-linked immunoabsorbant assay (ELISA) kits (Neogen Corporation, Lansing, MI, USA) according to Abbas et al. (2002, 2006). Triplicate sub-samples of ground maize (20 g) were extracted in 70% methanol (100 ml) for 3 min on a high-speed reciprocal shaker, clarified by centrifugation (10 min at 8000*g*), and the methanol extracts were analysed by ELISA. The limit of detection in this assay was $5\,ng\,g^{-1}$ total aflatoxin. The enumeration of *Aspergillus* propagule density and isolate characterization was assessed using selective media. Ground grain samples were homogenized in 0.2% w/v water agar, serially diluted and plated on MDRB agar. Colony-forming units (cfu) were calculated following 5 days of incubation. Thirty colonies per plot were transferred to CD-PDA (PDA with 0.3% β-cyclodextrin) and incubated for 5 days under continuous dark at 28°C, and evaluated for aflatoxin production based on colony pigmentation and colour change following exposure to ammonia vapours (Abbas et al. 2004).

Statistics

All field data, colonization assay, *Aspergillus* recovery and toxin phenotype, and aflatoxin contamination was analysed using PROC GLM of the Statistical Analysis System (SAS 2001). Mean separation was performed using Fisher's least significant difference.

Results and discussion

Survival of Aspergillus flavus *strain K49 in a water-dispersible granule*

A relatively high level of K49 survival was found following drying of the water-dispersible granule formulation with an initial count of $>3 \times 10^8\,cfu\,g^{-1}$ dried formulation (Table 1). No further loss of viability occurred during the storage of the formulated K49 following 11 months of storage at 4°C. This formulation provides a method to produce *A. flavus* inoculum months in advance of field applications and will enable initiation of larger-scale development trials required to optimize product definition and efficacy.

Field colonization by pin-bar assay

A similar final level of colonization of maize kernels by strain K49 introduced as formulated and unformulated conidia was observed at two locations in 2005 using the pin-bar inoculation assay (Table 2). The initial rate of K49 colonization of maize kernels observed at the Stoneville site (glyphosate-resistant hybrid) was faster than at the Elizabeth site (Bt hybrid). The Stoneville test was inoculated 10 days earlier than the Elizabeth test and meteorological conditions (warmer and drier air) may have influenced colonization. Further, *A. flavus* colonization in the non-inoculated control at the Stoneville site was greater than at the Elizabeth site. This difference may be attributed to the use of Bt hybrid (Elizabeth site), which lowers the incidence of the European cornborer (Dowd 2003). At the Elizabeth location a more rapid colonization of maize kernels was observed in ears inoculated with product 1 compared with the unformulated conidia at 5 and 7 days after inoculation, while at the Stoneville location both formulations elicited superior colonization compared with unformulated conidia at 7 days after inoculation.

Table 1. Recovery of *Aspergillus flavus* strain K49 produced in two different sized water-dispersible granules following initial drying and storage at 4°C.

	Product 1 (2.0 mm diameter)	Product 2 (1.2 mm diameter)
	Colony-forming units *Aspergillus flavus* g^{-1}	
Before drying	$3.09 \pm 0.08 \times 10^{8}$ [a]	$4.01 \pm 0.33 \times 10^{8}$
After drying	$3.12 \pm 0.35 \times 10^{8}$	$3.70 \pm 0.10 \times 10^{8}$
20-day storage	$2.57 \pm 0.15 \times 10^{8}$	$2.95 \pm 0.34 \times 10^{8}$
330-day storage	$3.90 \pm 0.36 \times 10^{8}$	$3.18 \pm 0.21 \times 10^{8}$

[a]Data are the means of three replicates and standard deviation (SD).

Superior colonization of strain K49 was observed with product 1 compared with product 2 at the Stoneville location at 5 days and at the Elizabeth location at 9 days after inoculation. There was no negative effect of formulation and formulation ageing (20 days in storage) on colonization by K49 conidia, and at certain early dates, colonization of formulated K49 was more aggressive than unformulated free conidia. Based on these preliminary results, product 1 (2.0 mm diameter water-dispersible granule) was chosen for application in the second year of field testing.

Recovery of **Aspergillus flavus** *from maize*

The number of log cfu of *A. flavus* recovered from ground maize ranged from 5.4 to 6.3 propagules g^{-1} grain, with the highest recovery from maize treated with a spray of unformulated conidia of K49 (Table 3). These levels of colonization were much higher than the *A. flavus* propagule counts found in maize in previous studies (Abbas et al. 2006) where recovery ranged from 3.4 to 4.4 for log cfu g^{-1} grain. The lowest proportion (4–6%) of toxigenic isolates was found in the formulated and unformulated spray applications of K49 (Table 3). In all other treatments a similar level of toxigenic isolates (50–71%) was observed and was not significantly affected by soil inoculation with either the non-toxigenic strain K49 or the toxigenic strain F3W4.

An approximate ratio of 24 : 1, 15 : 1 and 1 : 1, corresponding to recovered atoxigenic-to-toxigenic mean cfu, was determined for formulated spray, unformulated spray and remaining treatments, respectively. These results suggest that directed applications of the non-toxigenic strain K49 may be more effective

Table 2. Colonization of maize kernels following inoculation with K49 applied as water-dispersible granules or free conidia at two locations.

	Colonized kernels (%)[a]					
	Day 2	Day 5	Day 7	Day 9	Day 12	Day 14
Elizabeth (*BT*[b])						
Control	0	0c[c]	0.8c	2.1b	5.0b	7.5b
Product 1 (2.0 mm diameter)	0	54.1a	85.7a	100.0a	100.0a	98.1a
Product 2 (1.2 mm diameter)	0	51.1a	73.1b	97.2a	100.0a	98.1a
Unformulated conidia	0	34.4b	67.6b	100.0a	100.0a	92.0a
LSD ($Pr > 0.05$ level)		16.2	10.0	4.5	8.2	10.8
Stoneville (*RR*)						
Control	0.0b	0.9c	5.1c	9.4b	6.9b	23.9b
Product 1 (2.0 mm diameter)	0.6b	80.1a	98.7a	93.8a	98.5a	99.2a
Product 2 (1.2 mm diameter)	0.0b	37.5b	98.9a	94.5a	96.7a	93.6a
Unformulated conidia	17.2a	69.7a	85.7b	91.1a	96.0a	96.6a
LSD ($Pr > 0.05$ level)	4.7	14.55	12.0	8.9	5.2	14.3

[a]Mean of ten replicates sampled at each day.
[b]Bt, hybrid transformed to produce *Cry 1Ab* insecticidal protein; RR, hybrid modified for resistance to glyphosate.
[c]Values followed by the same letter are not differ significantly at the 95% confidence level, Fisher's protected Least Significant Difference (LSD) test.

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than soil treatments at enabling competitive displacement of native *Aspergillus* in colonization of maize ears. Previous research indicated that K49 colonization on maize ears could be enhanced by soil inoculation (Abbas et al. 2006); however, this was not observed in this study (Table 3). A possible explanation for this difference is that the soil inoculum of K49 was applied at the mid-silking (R2) stage in this study, whereas in the earlier study the inoculum was applied at the V6 stage of ontogeny. In studies on cotton, Cotty (1994) observed that 67% of the *A. flavus* recovered from cotton bolls were in the same vegetative compatibility group as the introduced non-toxigenic strain when applied as a soil inoculant compared with 46% as a spray inoculant and 25% in non-treated control plots. This indicated that a lower degree of establishment of the biological control strain was achieved by spray application in these Arizona field trials that probed cotton–*A. flavus* interaction with a different non-aflatoxigenic strain AF36.

The low recovery level of toxigenic *A. flavus* in maize that received spray treatments of K49 either formulated or unformulated indicates an apparent establishment, colonization and competitive displacement of toxigenic *Aspergillus* and could be attributed to the direct introduction of K49 to reproductive structures of maize.

Aflatoxin contamination

In these field studies a relatively high level of aflatoxin contamination was observed from natural infection in untreated control plots and in control plots where soil was inoculated with toxigenic F3W4 infested wheat.

Table 3. Recovery of *Aspergillus flavus* isolates and aflatoxin concentration from physiologically mature maize kernels as affected by inoculation treatments.

Treatment	*Apergillus flavus* ($\log_{10}$ cfu g^{-1})	Toxigenic isolates (%)	Aflatoxin concentration (μg kg^{-1})
No inoculant	5.5b,c	69a	428a,b
K49 wheat inoculant	5.7b	52a	44c
F3W4 wheat inoculant	5.7b	71a	635a
F3W4 plus K49 wheat inoculant	5.6b,c	50a	223b,c
F3W4 wheat inoculant plus K49 formulated spray	5.4c	4b	18c
F3W4 wheat inoculant plus K49 unformulated conidia spray	6.3a	6b	21c
LSD ($Pr > 0.05$ level)	0.2	42	287

Mean of three replicates, values followed by the same letter do not differ significantly at the 95% confidence level, Fisher's protected Least Significant Difference (LSD) test.

Respectively, 428 and 635 μg kg^{-1} aflatoxin was observed with a high variance among samples for these two control treatments (Table 3). However, where a soil application of K49 was made as infested wheat granules to plots that were either untreated or concurrently treated with a soil inoculation of F3W4, aflatoxin levels were reduced significantly ($p < 0.05$) to 44 and 223 μg kg^{-1} or by 90% and 65%, respectively.

In previous studies where K49 was introduced as a soil application, maize aflatoxin contamination was reduced by 58–76% relative to untreated plots when there was an abundant natural aflatoxin incidence (Abbas et al. 2006). When soil was inoculated with toxigenic isolate F3W4, co-inoculation with K49 on wheat-reduced aflatoxin contamination by 74–95% relative to aflatoxin concentrations in plots where soil was inoculated with F3W4 alone. In other studies, aflatoxin was reduced by 66 and 87% for two consecutive years in maize plots treated with equal mixtures of rice colonized by two different non-toxigenic *Aspergillus* species relative to aflatoxin levels in untreated maize plots (Dorner et al. 1999). The lower efficacy of soil applied K49 to reduce aflatoxin contamination observed in this study in comparison with the results of Abbas et al. (2006) may be related to the delayed application. These results confirm that aflatoxin contamination in maize can be controlled with soil applied treatments. Soil applications of K49 elicited a significant reduction in aflatoxin contamination; however, concentrations remained above regulatory limits for use of maize as food or feed stock (Table 3).

Spray treatments with either formulated or unformulated K49 conidial suspensions to plots where soil had been spiked with toxigenic F3W4 resulted in a 97% reduction in average aflatoxin concentration is attributed to spray inoculations with K49 in contrast to 65% from indirect soil application of K49 (Table 3). Specifically, spray application of K49 to reproductive maize structures in F3W4 soil-spiked plots significantly ($p < 0.05$) reduced aflatoxin levels to 18 or 21 μg kg^{-1} in comparison with 635 μg kg^{-1} in the control plot that received only the soil F3W4 application. The reduction in aflatoxin contamination when K49 was directly applied as a spray is consistent with observations of superior colonization by the non-toxigenic strain. In cotton, a grain application reduced aflatoxin contamination by 75% while no effect was observed when the non-toxigenic strain was applied as a spray to the reproductive tissues (Cotty 1994). While differences in formulation could account for discrepancy in efficacy between these spray treatments, these cotton trials were conducted in Arizona where environmental factors such as low relative humidity and high temperatures may have limited the success in establishment. Nevertheless, availability of multiple methods to apply aflatoxin biocontrol strains provides an option

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to choose the appropriate strategy for a particular cropping system.

Despite the widespread use and reliable success of solid inoculants in cotton and peanuts, the strategy has not been commercially adopted for aflatoxin control in maize. Due to anatomical differences between maize and either cotton or peanuts, soil inoculation may not be the most effective biological control strategy for maize. Difference in mean per cent reduction between soil and spray treatments in this study could be attributed to the application method. While fruiting structures in peanuts are below the soil surface, a soil application is directed near the area of infection. In addition, the reproductive structures in cotton are distributed from the third to the twelfth node and are relatively close to the soil surface. By contrast, maize is a fast-growing, relatively tall monocot species producing reproductive structures >1.0 m from the soil surface. Although soil and plant residues residing on the soil surface is typically the major reservoir for *A. flavus* in the field environment, the actual source of infection of maize cobs is diverse and is dependent on the environment. The infective propagules may have been derived from aerial dispersal of soil propagules in close proximity or long range aerial transport from a distant location. In addition, insects that cause physical or mechanical damage to maize ear are often associated with *Aspergillus* ear rot. For colonization and subsequent displacement of indigenous toxigenic strains on maize, a high level of inoculum is generally required with a transfer mechanism for soil-applied biological control agents to reach and be maintained on the aerial target sites (i.e., maize ears) (Dorner et al. 1999). In the case of a direct spray application, this requirement may be unnecessary as was demonstrated in this study.

The use of wheat inoculants to control aflatoxin in maize poses other drawbacks such as (1) transport and application of a solid matrix may be difficult for commercial use when the crop is at later stages of growth, (2) environmental and climatic factors such as wind, rain and humidity may limit or delay conidial dispersal from granular point sources to aerial regions of maize, and (3) the potential and associated risk of prolonged increase in *A. flavus* propagules in the air arising from ongoing sporulation on the applied grains in the field may raise health and safety issues.

Although there was no significant difference in aflatoxin levels from the formulated and unformulated conidia of K49, the formulated material was eleven months old and demonstrated equivalent efficacy as freshly harvested conidia. As application of freshly generated biological control agents is unlikely to be a viable commercial option, stable formulations to effectively deliver these fungi such as the water-dispersible granule described herein, present a commercially feasible option for controlling aflatoxin in maize and other crops with susceptible aerial infection courts. We note that in this study the formulation, presented as a new delivery system, was applied at $9\,\mathrm{kg\,ha^{-1}}$, whereas grain based soil inoculants have been applied at rates from 20 to $200\,\mathrm{kg\,ha^{-1}}$ (Cotty 1994; Dorner et al. 1998; Abbas et al. 2006). Further optimization of formulation and method of spray application may reduce the amount of formulation required for aflatoxin control. While only one biological active ingredient was incorporated in the water-dispersible granule, the composition could be modified to accommodate other biological actives. The dispersible granule composition may make it possible to co-deliver non-toxigenic *A. flavus* with a biological ingredient that is active against *Lepidotera* species such as *Bacillus thuringiensis* for control of the European corn borer larvae.

A simple preparation method for a water-dispersible granule formulation containing a non-toxigenic biological control *A. flavus* strain (K49) is presented. Similar levels of colonization and reduction in aflatoxin are found between spray applications of formulated and unformulated conidia. The significance of this finding is that a suitable biological control product can be developed and applied using conventional application technologies to mitigate aflatoxin contamination in maize. The excellent reduction in aflatoxin levels and apparent establishment of the applied *A. flavus* strain supports the hypothesis that a direct application to aflatoxin susceptible regions on maize may be the most effective method for reducing aflatoxin contamination. Further field testing in multiple locations will be required to proceed with assessing the real impact of this strategy for delivering non-toxigenic strains of *A. flavus* for biological control of aflatoxin contamination in maize and perhaps other crops.

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